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Assessment of *Pseudomonas syringae* pv. *tagetis* as a biocontrol agent for Canada thistle

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Growth chamber and field experiments were conducted to assess the potential of *Pseudomonas syringae* pv. *tagetis* (Pst) as a biocontrol agent for Canada thistle. Silwet L-77, an organosilicone surfactant, was required to facilitate Pst penetration into Canada thistle leaves. Growth chamber experiments indicated that maximum Pst populations inside leaves were obtained with a Silwet L-77 concentration of 0.3% (v/v) or greater. High Pst populations (10^9 colony-forming units [cfu] per gram fresh weight) were found in leaves 48 h after treatment with 10^8 or 10^9 cfu ml^{-1} Pst plus Silwet L-77 (0.3%, v/v). In growth chamber experiments, foliar application of Pst (10^9 cfu ml^{-1}) plus Silwet L-77 (0.3%, v/v) on 4- to 5-wk-old Canada thistle reduced shoot dry weight by 52% (measured 14 d after treatment) and chlorophyll content of emerging leaves by 92% (measured 10 d after treatment). In field trials conducted in 1999 and 2000, Pst (10^9 cfu ml^{-1}) plus Silwet L-77 (0.3%, v/v) were applied at 700 L ha^{-1}, and the method of application (paint gun, backpack sprayer, boom) and the number of applications (one or two separated by 14 d) were examined. Averaged over 2 yr, two applications with a backpack sprayer resulted in 67% disease incidence (apical chlorosis) of treated plants measured 4 wk after the initial treatment (WAIT). At the time of flower bud formation (8 WAIT), there was little or no disease incidence, 31% reduction in plant height, 81% reduction in number of flower buds, and 20% reduction in shoot survival during 1999 but no effect on survival in 2000.

Nomenclature: Canada thistle, *Cirsium arvense* (L.) Scop. CIRAR; soybean, *Glycine max* L. 'Lambert', 'Kato'.

Key words: Bacterial population dynamics, bioherbicide, biological control, organosilicone surfactant, tagetitoxin.

Pseudomonas syringae pv. *tagetis* (Pst) causes leaf spot and apical chlorosis in various Asteraceae, including the weeds Canada thistle and common ragweed (*Ambrosia artemisiifolia* L.) (Gulya et al. 1982; Johnson and Wyse 1991, 1992; Johnson et al. 1996; Rhodehamel and Durbin 1985; Styer and Durbin 1982). Apical chlorosis elicited by Pst is due to the production of tagetitoxin, a non-host-specific toxin (Durbin 1990; Lukens and Durbin 1985). Tagetitoxin is translocated to emerging leaves, where it inhibits plastidic RNA polymerase III, which in turn prevents chloroplast biogenesis (Mathews and Durbin 1990, 1994; Steinberg et al. 1990).

Canada thistle is a serious weed control problem in organic soybean production in Minnesota (Carmen Fernholz, personal communication). Previous research indicated that Pst might have potential as a biological agent for controlling Canada thistle in soybean. Multiple (three to five) inundative foliar applications of Pst (10^8 to 10^9 colony-forming units [cfu] ml^{-1}) plus the organosilicone surfactant Silwet L-77 (0.05 to 0.2%, v/v) elicited disease incidence (apical chlorosis) and reduced Canada thistle survival, height, and seed production (Hoeft et al. 2001; Johnson and Wyse 1991, 1992; Johnson et al. 1996). Single inundative foliar applications of Pst appear to be less effective. In a greenhouse study a single application of Pst (5×10^8 cfu ml^{-1}) plus Silwet L-77 (0.3%, v/v) on 2-wk-old Canada thistle resulted in disease incidence (chlorosis) but had no effect on shoot dry weight measured 5 wk after the treatment (Bailey et al. 2000).

Inundative foliar application of Pst plus Silwet L-77 also causes disease symptoms in certain non-host species, such as woollyleaf bursage [*Ambrosia grayi* (A.Nels.) Shinners, AMBGR] (Sheikh et al. 2001), common cocklebur (*Xanthium strumarium* L., XANST) (Abbas et al. 1995), and houndstongue (*Cynoglossum officinale* L., CYWOF) (Zidack et al. 2000). Multiple (four) applications of Pst plus Silwet L-77 (0.25%, v/v) to woollyleaf bursage resulted in high disease incidence and a reduction in stem density. For common cocklebur, foliar application of Pst (5×10^8 cfu ml^{-1}) reduced shoot biomass by approximately 60%. Field studies conducted in 1997 indicated that Pst applied with Silwet L-77 caused a significant reduction in the survival of houndstongue plants.

Research involving both growth chamber and field experiments was conducted to evaluate the potential of Pst as a biocontrol agent for Canada thistle in soybean. Growth chamber experiments were conducted to (1) determine the concentration of Silwet L-77 required for the maximum penetration of Pst into Canada thistle leaves, (2) characterize the dynamics of Pst populations in treated leaves of Canada thistle and soybean after application, (3) evaluate disease incidence (apical chlorosis) in emerging leaves of Canada thistle as a function of Pst concentration applied, and (4) quantify the effects of inundative foliar application on Canada thistle and soybean shoot biomass. Field trials were conducted to examine the ability of foliar application of Pst (10^9 cfu ml^{-1}, 0.3% [v/v] Silwet L-77) to control Canada

thistle growing within the row of soybean. Different methods of foliar application (backpack sprayer, paint gun, boom) and number of Pst applications (one or two) were investigated. The number of applications was limited to two because it was felt that this would be the upper limit of what would be acceptable to most farmers. Efficacy of Pst applications was evaluated in terms of disease incidence (apical chlorosis) and effect on Canada thistle shoot height, number of flower buds, and survival.

Materials and Methods

Growth Chamber Experiments

Plant Material

Canada thistle seeds[1] were planted 0.5 cm deep in trays (16 × 24 cm) containing Sunshine Mix #1.[2] After approximately 2 wk, Canada thistle plants were transferred to pots (8.5 by 8.5 or 13 by 13 cm). Soybean 'Lambert' seeds were planted 1.5 cm deep in pots (8.5 by 8.5 cm) containing Sunshine Mix #1. Canada thistle and soybean were grown in a growth chamber with day and night temperatures of 25 and 20 C, respectively, and a 16-h photoperiod (photosynthetic photon flux [PPF], 500 μmol m^{-2} s^{-1}). Pst was applied to soybean 8 to 10 d after planting, when plants were approximately 10 cm tall, and unifoliates were fully emerged and trifoliates were just emerging. Pst was applied to Canada thistle in the eighth- to ninth-leaf stage (approximately 10 cm tall), which was 2 to 3 wk after emergence or 4 to 5 wk after planting. Plants were watered daily with 0.1× Hoagland's solution (Hoagland and Arnon 1950).

Pst Culture

Pst, strain 1-502a, originally isolated from infected Canada thistle was maintained at 4 C by monthly subculturing on agar plates containing a modified (glycerol substituted for sucrose) 523 medium (Kado and Heskett 1970). A 125-ml volume of modified 523 medium was inoculated from the culture plate and grown for 24 h at 23 C on a rotary shaker (300 rpm min^{-1}). The final Pst concentration in the culture was approximately 5×10^9 cfu ml^{-1}. Inoculum density was adjusted based on turbimetric measurements (600 nm) to obtain a bacterial population of 10^9 cfu ml^{-1}. In some experiments the Pst culture was pelleted by centrifugation (4,000 × *g,* 15 min at 4 C) in a sterile centrifuge bottle (250 ml) and resuspended in 100 ml of sterile, distilled water. Serial dilutions of the cells were made in sterile water to obtain suspensions containing 10^8 to 10^6 cfu ml^{-1}. Silwet L-77[3] was added to the Pst suspensions just before application to obtain final concentrations of 0.15, 0.30, 0.45, or 0.70% (v/v). For all experiments, except those to determine optimum surfactant concentration, Silwet L-77 was added to Pst spray suspensions at 0.3% (v/v).

Pst Application

Pst was applied to Canada thistle and soybean plants at a pressure of 242 kPa in a ventilated fume hood using a handheld paint sprayer.[4] Potted plants were placed in a tray (25 by 52 cm), and two passes of approximately 3 s each were made with the sprayer positioned approximately 25 cm above the tray at a 45° angle. The tray was turned 180° between passes. The estimated volume per unit area applied using this application method was 1.7×10^3 L ha^{-1}. After spraying, the treated plants were immediately placed back in the growth chamber.

Growth Response

Canada thistle and soybean plants, approximately 10 cm tall and grown as described earlier, were sprayed with 10^9 cfu ml^{-1} Pst plus Silwet L-77 (0.3%, v/v). Shoots were harvested 2 wk after treatment, and dry weights were obtained after drying plant material for 72 h at 95 C. The data represent the results of experiments repeated once with eight replicates per treatment. Populations were normally distributed and exhibited homogeneous variance. Data were analyzed by analysis of variance (ANOVA). A Tukey test was used for pairwise multiple comparisons ($P = 0.05$).

Pst Populations in Leaves

Canada thistle and soybean plants, approximately 10 cm tall and grown as described earlier, were sprayed with Pst (10^6 to 10^9 cfu ml^{-1}) plus Silwet L-77 (0.3%, v/v). For Canada thistle, two mature leaves (fifth or sixth leaf of plants in the eighth- or ninth-leaf stage at the time of treatment) were sampled from different plants at intervals (15 min to 240 h) after Pst application. For soybean, two unifoliates from different plants were sampled at the same time interval. Leaves were surface sterilized by sequentially rinsing with vigorous agitation for 20 s each in the following five solutions contained in petri dishes: 70% ethanol, sterile water, 70% ethanol, sterile water, and sterile water. This rinse technique was as effective as a 3 min rinse in 10% bleach (0.5% sodium hypochlorite) for removing epiphytic bacteria (Henis et al. 1982). Leaves were blotted dry, and eight leaf disks were punched out with a sterile paper punch (0.5-cm diameter) and weighed. Disks were taken from both sides of the leaf approximately equidistant between the midvein and the leaf edge. Leaf disks were ground in 1.5 ml sterile water using a mortar and pestle. Serial dilutions of the extract were made in sterile water, and 100 μl of each dilution was plated in petri dishes containing modified 523 agar. Plates were incubated at 23 C for 48 h, and individual Pst colonies were counted. Pst population data represent the mean ± standard error of experiments repeated three or four times. Data, expressed as cfu g fresh wt^{-1}, were $\log_{10}$ transformed before calculation of population statistics.

Chlorophyll Determination

Canada thistle plants, approximately 10 cm tall and grown as described earlier, were sprayed with Pst (10^6 to 10^9 cfu ml^{-1}) plus Silwet L-77 (0.3%, v/v). The chlorophyll content of the two youngest Canada thistle leaves that emerged after Pst treatment was determined as an index of disease development. Chlorophyll was measured 10 d after treatment. Excised leaves were weighed and ground in liquid nitrogen using a mortar and pestle. The pulverized leaf tissue was added to 80% acetone (0.5 g leaf tissue 5 ml^{-1}), vortexed, and incubated for 30 min in the dark at room temperature. The solution was vortexed and centrifuged (3,000 × *g,* 10 min), and the absorbance of the supernatant was measured at 663, 652, and 645 nm to determine chlo-

rophyll (Arnon 1949). Leaf chlorophyll data represent the mean of experiments repeated six times with four or eight replicates per experiment ($n = 40$) and are expressed as milligrams of chlorophyll per gram fresh weight. Data were analyzed by ANOVA. Dunn's test was used for pairwise multiple comparisons ($P = 0.05$).

Field Trials

Pst Culture

Pst, strain 1-502a, was maintained at 4 C by monthly subculturing on agar plates, as described earlier. A loop of Pst cells from the culture plate was used to inoculate 100 ml of tryptic soy broth (TSB) in a 250-ml Erlenmeyer flask, and the culture was allowed to grow overnight on a rotary shaker (23 C, 300 rpm). An aliquot of this culture (50 ml) was used to inoculate 1 L of TSB medium in a 2.8-L Fernbach flask. This culture was allowed to grow overnight on the rotary shaker. The next morning 50-ml aliquots were transferred to ten Fernbach flasks containing 1 L of TSB medium. These cultures were grown for 24 h on the rotary shaker, reaching a stationary phase population of approximately 5×10^9 cfu ml^{-1}. Appropriate dilutions were made in sterile distilled water to yield a Pst concentration of 10^9 cfu ml^{-1}. Silwet L-77 (0.3%, v/v) was added to the Pst suspensions just before application.

Treatments and Experimental Design

Experiments to evaluate the effects of inundative Pst applications on controlling Canada thistle growing within the row of soybean were conducted on a farm in Madison, MN. Based on the results of the growth chamber experiments described earlier, field trials were conducted using foliar applications of 10^9 cfu ml^{-1} Pst plus Silwet L-77 (0.3%, v/v). Apical chlorosis of treated plants was used to evaluate disease incidence. The field, a Webster clay loam (fine-loamy, mixed, mesic type Haplaquoll), contained a dense natural infestation of Canada thistle. Soybean 'Kato' seed were planted at a density of 28 plants m^{-1} in 76-cm rows that were 9 m long. Soybean was grown using organic-farming practices that comply with Minnesota organic certification agency standards. Weeds were controlled between rows by two mechanical cultivations. The experimental design was a randomized complete block. Each plot consisted of four, 9-m rows with a 1.5-m alley between plots. In each plot ten Canada thistle plants within the soybean row were randomly selected and marked with a plastic tag around the base of the stem. Treatments were the method of application (paint gun,[4] backpack sprayer,[5] and boom[6]) and the number of applications (one or two with the second application 14 d after the first). For the backpack sprayer treatment, Pst solutions were applied at 276 kPa. For both the boom and the paint gun treatments, Pst solutions were applied at 242 kPa. In 2000 another treatment, two applications of Silwet L-77 (0.3%, v/v) with a backpack sprayer, was added to assess the effects of the surfactant on Canada thistle height, survival, and flower bud formation. For all application methods, Silwet L-77 (0.3%, v/v) alone or with Pst (10^9 cfu ml^{-1}) was applied at 700 L ha^{-1}. Each treatment was replicated four times. For all treatments, the first Pst application was made when Canada thistle plants were approximately 7 cm tall. For half of the treatments, a second application was made 2 wk after the first.

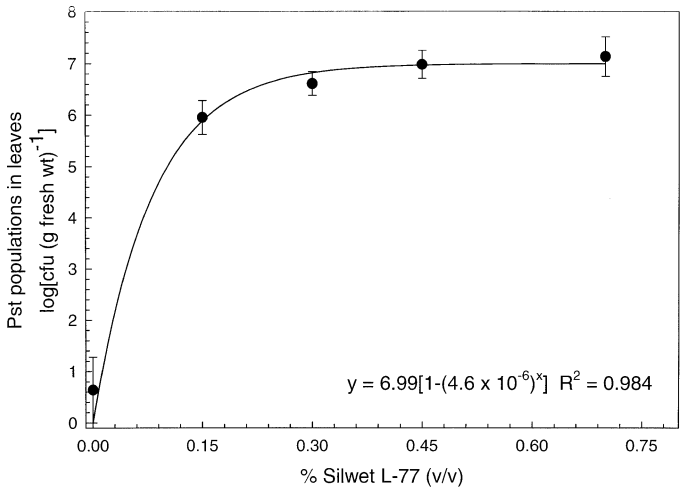

FIGURE 1. *Pseudomonas syringae* pv. *tagetis* (Pst) populations in Canada thistle leaves sprayed with 10^9 cfu ml^{-1} Pst plus variable concentrations of Silwet L-77 (0.15 to 0.7% v/v). Pst populations were measured 15 min after application. Values represent means ± SE of four experiments ($n = 4$) conducted in a growth chamber. The exponential equation describing the data was significant at $P < 0.001$.

In 1999 and 2000 soybeans were planted on June 2 and May 25, respectively. In 1999 the first Pst application was made on June 25, and the second, where required, was made on July 9. For both dates, air temperature at the time of application was approximately 30 C. In 2000 the first Pst application was made on June 29 and the second, where required, was made on July 13. Air temperatures at the time of the first and second Pst applications were approximately 24 and 29 C, respectively. For the marked plants in each plot, disease incidence (measured as % plants exhibiting chlorosis) was recorded twice—4 and 8 wk after initial treatment (WAIT). Plant height, number of flower buds per plant, and plant survival measurements were taken of the marked plants at the time of flower bud formation, which was 8 WAIT.

Statistical Analysis

All data obtained in field trials were normally distributed and exhibited homogeneous variance. Data were analyzed by ANOVA, and a Tukey test ($P = 0.05$) was used for pairwise multiple comparisons.

Results and Discussion

Growth Chamber Experiments

Maximum Pst populations of approximately 10^7 cfu g fresh wt^{-1}, measured 15 min after application, were obtained when Pst was applied with a Silwet L-77 concentration of 0.3% (v/v) or greater (Figure 1). Without the addition of Silwet L-77 to the application medium, very low levels of Pst (approximately 10 cfu g fresh wt^{-1}) were found in leaves. Previous studies demonstrated the necessity of adding Silwet L-77, an organosilicone surfactant, to the application suspension to facilitate the entry of bacteria into leaves (Zidack and Backman 1996; Zidack et al. 1992). Silwet L-77 facilitates the movement of bacteria into stomata

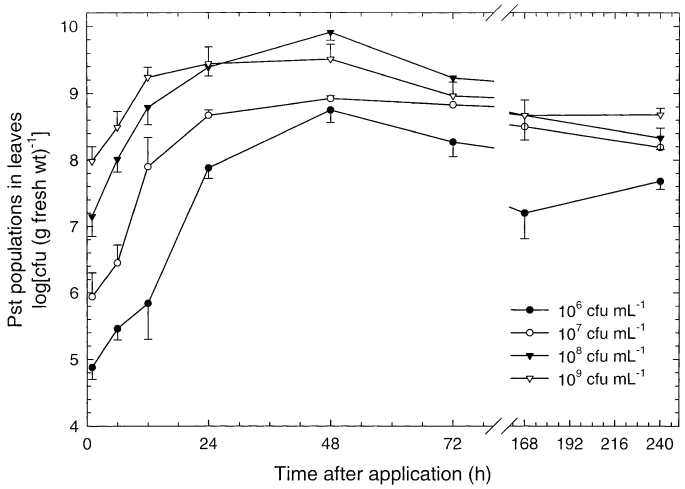

FIGURE 2. *Pseudomonas syringae* pv. *tagetis* (Pst) populations in Canada thistle leaves 1 to 240 h after spraying with Silwet L-77 (0.3%, v/v) plus 10^6 to 10^9 cfu ml^{-1} Pst. Values represent means ± SE of four experiments (n = 4) conducted in a growth chamber.

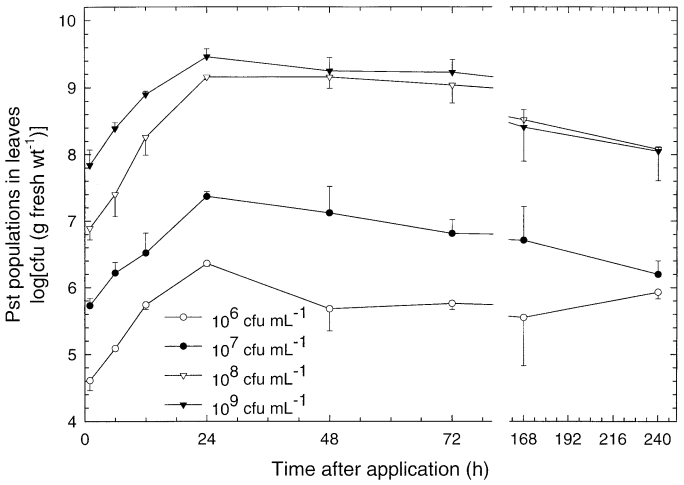

FIGURE 3. *Pseudomonas syringae* pv. *tagetis* (Pst) populations in soybean leaves 1 to 240 h after spraying with Silwet L-77 (0.3%, v/v) plus 10^6 to 10^9 cfu ml^{-1} Pst. Values represent means ± SE of three experiments (n = 3) conducted in a growth chamber.

and hydathodes because of its low surface tension (20 dynes cm^{-1}) (Field and Bishop 1988; Neumann and Prinz 1974; Stevens et al. 1991). Our results are consistent with the previous report that a Silwet L-77 concentration of 0.2% (v/v) or greater was required for maximum penetration of *Pseudomonas syringae* pv. *phaseolicola* into bean (*Phaseolus vulgaris* L.) leaves (Zidack et al. 1992).

The dynamics of Pst populations inside Canada thistle leaves were evaluated at intervals between 1 to 240 h after application (Figure 2). Initial populations inside leaves measured 1 h after treatment were linearly related (r = 0.99) to the Pst concentration applied (10^6 to 10^9 cfu ml^{-1}). During the initial 24 h after application, rapid exponential growth occurred in intercellular spaces where nutrients and moisture are available. Within 24 to 48 h after treatment, Pst populations in leaves reached maximum levels. High Pst populations (10^9 cfu g fresh wt^{-1}) were found in leaves 48 h after treatment with 10^8 or 10^9 cfu ml^{-1} Pst. For all treatments, Pst populations inside leaves 10 d after treatment were greater than populations measured 1 h after treatment. The population dynamics of Pst in soybean leaves were similar to those in Canada thistle when Pst was applied at 10^8 or 10^9 cfu ml^{-1} (Figure 3). Pst populations in soybean leaves 48 h and 10 d after treatment with 10^9 cfu ml^{-1} Pst were approximately 10^9 and 10^8 cfu g fresh wt^{-1}, respectively. The most noticeable difference in the population dynamics of Pst in Canada thistle and soybean was the significantly lower population found in soybean leaves treated with 10^6 or 10^7 cfu ml^{-1} Pst. Most likely, this is the result of the hypersensitive response that occurred in soybean, a non-host species.

The effect of Pst concentration (10^6 to 10^9 cfu ml^{-1}) on disease incidence in Canada thistle was determined by measuring the chlorophyll content of leaves that emerged after Pst treatment (Figure 4). Silwet L-77 (0.3%, v/v) alone had no effect on the chlorophyll content of emerging leaves. Pst concentrations of 10^6 cfu ml^{-1} reduced the chlorophyll content of emerging leaves by 44%. When Pst was applied at 10^8 or 10^9 cfu ml^{-1}, chlorophyll content of newly emerged leaves was reduced by approximately 90%. Foliar application of Pst (10^9 cfu ml^{-1}) plus Silwet L-77 (0.3%, v/v) did not elicit disease symptoms (apical chlorosis) in soybean, a non-host species.

The effects of foliar application of 10^9 cfu ml^{-1} Pst plus Silwet L-77 (0.3 %, v/v) on shoot growth of Canada thistle and soybean were examined (Table 1). Silwet L-77 (0.3%, v/v) applied alone caused small water-soaked spots on leaves of both species that became necrotic after 24 h. However, this injury was relatively minor and did not have a significant effect on shoot dry weight. For soybean, in addition to Silwet-induced lesions, there were also small necrotic lesions on treated leaves because of the hypersensitive response elicited by Pst in this non-host species. However, inundative foliar application of Pst (10^9 cfu ml^{-1}) plus Silwet L-77 did not have a significant effect on soybean shoot dry weight measured 2 wk after treatment. Treatment with 10^9 cfu ml^{-1} Pst plus Silwet L-77 (0.3%, v/v) reduced shoot dry weight of Canada thistle by 52%, when compared with the control.

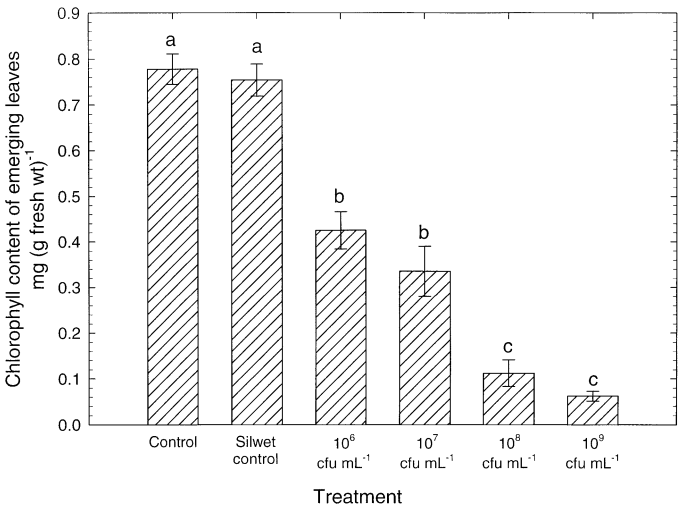

FIGURE 4. Chlorophyll content of emerging leaves of Canada thistle measured 10 d after plants were sprayed with Silwet L-77 (0.3%, v/v) or Silwet L-77 (0.3%, v/v) plus Pst (10^6 to 10^9 cfu ml^{-1}). Values represent means ± SE of seven experiments (n = 40) conducted in a growth chamber. Bars with different letters are significantly different ($P < 0.05$), according to Dunn's test.

TABLE 1. Effect of inundative application of Silwet L-77 (0.3%, v/v) alone or with 10^9 cfu ml^{-1} *Pseudomonas syringae* pv. *tagetis* (Pst) on shoot dry weight of Canada thistle [*Cirsium arvense* (L.) Scop.] and soybean (*Glycine max* L.) grown in a growth chamber.

Treatment	Shoot dry weight[a]	
	Canada thistle	Soybean
	g	
Control (nontreated)	1.44 a	1.28 a
Silwet L-77 (0.3%) control	1.23 a	1.32 a
Silwet L-77 (0.3%) plus 10^9 cfu ml^{-1} Pst	0.59 b	1.32 a
P value	< 0.001	0.866

[a] Shoot dry weight measured 2 wk after application. A pairwise comparison of the means at a 95% confidence level was accomplished by analysis of variance. Different letters indicate a significant difference between treatments within a species.

None of the Canada thistle plants treated with Pst (10^9 cfu ml^{-1}) plus Silwet L-77 (0.3%, v/v) died.

Field Trials

For both 1999 and 2000, disease incidence was greater when measured 4 WAIT than at the time of flower bud formation, which was 8 WAIT (Table 2). In 1999 the highest levels of disease incidence measured 4 WAIT were obtained with two applications with a backpack sprayer or a paint gun, where 71 and 51% of treated plants exhibited chlorosis. In 2000 one or two applications with a backpack sprayer resulted in maximum disease incidence 4 WAIT with 50 and 63% of the treated plants exhibiting chlorosis, respectively. For both 1999 and 2000, a second Pst application resulted in no significant increase in disease incidence measured 4 WAIT, with one exception. The exception occurred in 1999 where a second Pst application with a backpack sprayer approximately doubled the number of plants exhibiting chlorosis. When disease incidence was measured at the time of flower bud formation (8 WAIT), few, if any, plants exhibited chlorosis for any application method (Table 2). In most cases the chlorotic leaf tissue observed 4 WAIT had re-greened, and new growth did not exhibit chlorosis.

For both years, the mean height of nontreated Canada thistle at the time of flower bud formation was approximately 57 cm (Table 3). Two applications of Silwet L-77 (0.3%, v/v) with a backpack sprayer caused minor leaf injury but did not have a significant effect on shoot height. In general, inundative foliar application of Pst (10^9 cfu ml^{-1}) plus Silwet L-77 (0.3%, v/v) had relatively small effects or no effect on plant height. In 1999 three treatments (one or two applications with a backpack sprayer, two applications with a paint gun) reduced plant height by approximately 30%. Other treatments did not cause a significant reduction in plant height. In 2000 none of the treatments caused a significant reduction in plant height compared with the nontreated control. For both years, a second Pst application by any method did not result in a significant reduction in plant height beyond that caused by a single application.

For both 1999 and 2000, nontreated Canada thistle plants exhibited an average of 13 flower buds per plant (Table 4). Two applications of Silwet L-77 (0.3%, v/v) with a backpack sprayer had no effect on the number of flower buds. In 1999 the number of flower buds per plant was reduced by one or two applications with the backpack sprayer and two applications with a boom. In 2000 all treatments except a single application with the boom caused a significant reduction in the number of flower buds. Averaged over both years, a single application with a backpack sprayer reduced the number of flower buds by approximately 80%. For both 1999 and 2000, a second Pst application by any method resulted in no additional reduction in the number of flower buds.

For both 1999 and 2000, approximately 10% of the nontreated Canada thistle plants did not survive until the time of flower bud formation (Table 5). Applying Silwet L-77 (0.3%, v/v) without Pst had no effect on Canada thistle shoot survival. With the exception of two applications with a backpack sprayer in 1999, which reduced survival by an additional 20% compared with the nontreated control, none of the Pst treatments caused a significant reduction in survival.

One objective of this research was to evaluate whether different application methods influenced the efficacy of Pst as a biocontrol agent for Canada thistle. There was considerable year-to-year variability in the efficacy of the application methods examined. However, if a comparison is made on the basis of the results averaged over both years, the backpack sprayer appears to be the best application method.

TABLE 2. Effect of the method of *Pseudomonas syringae* pv. *tagetis* (Pst) (10^9 cfu ml^{-1}) application and the number of applications on the percentage of Canada thistle [*Cirsium arvense* (L.) Scop.] plants exhibiting chlorosis in soybean (*Glycine max* L.).

Treatment[a]	Number of applications[b]	% Plants exhibiting chlorosis			
		1999		2000	
		4 WAIT[c]	8 WAIT[c,d]	4 WAIT[c]	8 WAIT[c,d]
Backpack sprayer	1	38 bc	3 a	50 ac	3 a
Backpack sprayer	2	71 a	7 a	63 a	9 a
Paint gun	1	25 bc	0 a	21 b	0 a
Paint gun	2	51 ac	0 a	26 bc	0 a
Boom	1	18 b	3 a	13 b	0 a
Boom	2	20 b	0 a	14 b	0 a
P value		< 0.001	0.219	< 0.001	0.193

[a] All treatments contained 10^9 cfu ml^{-1} Pst plus Silwet L-77 (0.3%, v/v).
[b] Second application was made 2 wk after the first application.
[c] Abbreviation: WAIT, weeks after initial treatment.
[d] At the time of flower bud formation.

TABLE 3. Effect of the method of *Pseudomonas syringae* pv. *tagetis* (Pst) (10^9 cfu ml^{-1}) application and the number of applications on the shoot height of Canada thistle [*Cirsium arvense* (L.) Scop.] in soybean (*Glycine max* L.).

Treatment[a]	Number of applications[b]	Shoot height[c]	
		1999	2000
		cm	
Control (nontreated)	—	56 a	58 ab
Backpack sprayer (0.3% Silwet alone)	2	ND[d]	61 a
Backpack sprayer	1	41 bcd	47 ac
Backpack sprayer	2	36 be	43 bc
Paint gun	1	45 ade	52 ac
Paint gun	2	43 bcd	49 ac
Boom	1	52 ac	50 ac
Boom	2	50 ad	47 ac
P value		<0.001	0.024

[a] Except for the control (nontreated), all treatments contained Silwet L-77 (0.3%, v/v).
[b] Second application was made 2 wk after the first application.
[c] Shoot height was determined at the time of flower bud formation, which was 8 wk after initial treatment.
[d] Abbreviation: ND, not determined.

In general, backpack sprayer application resulted in high levels of disease incidence (measured 4 WAIT) and caused an 80% reduction in the number of flower buds. In addition, the backpack sprayer (two applications) was the only application method to cause a significant reduction in shoot survival (20%), but this was observed for only 1 yr (1999).

Another objective of this study was to evaluate whether a second Pst application applied 2 wk after the first increased Pst efficacy on Canada thistle. When evaluated over 2 yr, the data do not show a consistent benefit of a second Pst application for the parameters measured. In 1999 a second backpack sprayer application increased disease incidence expressed 4 WAIT and decreased survival. In 2000 a second application by any method did not result in a significant increase in efficacy for any of the parameters measured.

The most significant and consistent effect of Pst application was the reduction in the number of flower buds. A single Pst application with a backpack sprayer reduced the number of flower buds per plant by approximately 80%. A similar reduction of Canada thistle seed heads per plant (approximately 75% measured 69 d after planting) was reported by Hoeft et al. (2001) when using a backpack sprayer and a more intensive Pst application regime (three applications of Pst [10^9 cfu ml^{-1}] applied over 12 d). Although a strategy that primarily reduces seed production would not be acceptable for Canada thistle control in annual crops, it may have an application for reducing seed production in noncropland (conservation reserve program—CRP land, roadsides, pastures, range). This would be particularly true if a single inundative Pst application resulted in an infection that carried over to subsequent years and hence continued to reduce seed production. However, this has not been examined.

Inundative foliar application of Pst (10^9 cfu ml^{-1}) plus Silwet L-77 (0.3%, v/v) does not provide effective control of Canada thistle shoot growth or survival. For the 2-yr study, only three treatments caused a significant (approximately 30%) reduction in plant height. For both years, only one treatment (two applications with a backpack sprayer) applied in 1999 reduced Canada thistle survival, and this was by only 20%. Clearly, the limited and inconsistent effects of inundative Pst application on Canada thistle shoot growth and survival would not favor adoption of this biocontrol strategy.

The efficacy of inundative Pst application on Canada thistle survival reported by Hoeft et al. (2001) was greater than

TABLE 4. Effect of the method of *Pseudomonas syringae* pv. *tagelis* (Pst) (10^9 cfu ml^{-1}) application and the number of applications on the number of flower buds per Canada thistle [*Cirsium arvense* (L.) Scop.] plant in soybean (*Glycine max* L.).

Treatment[a]	Number of applications[b]	Flower buds[c]	
		1999	2000
		number $plant^{-1}$	
Control (nontreated)	—	13 a	13 a
Backpack sprayer (0.3% Silwet alone)	2	ND[d]	13 a
Backpack sprayer	1	3 bd	3 b
Backpack sprayer	2	2 be	3 b
Paint gun	1	8 acd	6 bc
Paint gun	2	7 ade	4 bc
Boom	1	12 ac	9 ac
Boom	2	6 bce	4 bc
P value		< 0.001	< 0.001

[a] Except for the control (nontreated), all treatments contained Silwet L-77 (0.3%, v/v).
[b] Second application was made 2 wk after the first application.
[c] Number of flower buds per plant was determined at 8 wk after initial treatment.
[d] Abbreviation: ND, not determined.

TABLE 5. Effect of the method of *Pseudomonas syringae* pv. *tagelis* (Pst) application (10^9 cfu ml^{-1}) and the number of applications on the survival of Canada thistle [*Cirsium arvense* (L.) Scop.] in soybean (*Glycine max* L.).

Treatment[a]	Number of applications[b]	% Survival[c]	
		1999	2000
Control (nontreated)	—	88 a	90 a
Backpack sprayer (0.3% Silwet only)	2	ND[d]	88 a
Backpack sprayer	1	80 a	88 a
Backpack sprayer	2	68 b	85 a
Paint gun	1	85 a	95 a
Paint gun	2	80 a	90 a
Boom	1	85 a	93 a
Boom	2	85 a	93 a
P value		< 0.001	0.645

[a] Except for the control (nontreated), all treatments contained 0.3% (v/v) Silwet L-77.
[b] Second application was made 2 wk after the first application.
[c] Percentage survival of shoots was determined at the time of flower bud formation, which was 8 wk after initial treatment.
[d] Abbreviation: ND, not determined.

that observed in this study. Averaged over 2 yr, Canada thistle survival, compared with the control, was reduced by approximately 55% when measured 105 d after planting. This may be because of the more intensive Pst application regime (three applications of Pst [10^9 cfu ml^{-1}] within a 12-d period) used by Hoeft et al. (2001). However, it is also possible that the greater effect of Pst was partly because of the stress imposed by imazethapyr (0.07 kg ai ha^{-1} plus 0.25% [v/v] nonionic surfactant) applied to Canada thistle 9 d before Pst applications were initiated. Because of this treatment, Canada thistle plants were stunted and exhibited apical chlorosis at the time Pst applications were initiated (Hoeft 1998). It is not clear to what extent the stress imposed by the imazethapyr treatment may have made Canada thistle more susceptible to the additional stress imposed by three subsequent Pst applications.

The primary challenge to developing Pst as a biocontrol agent for Canada thistle is the need to increase its effectiveness in reducing plant growth and survival. One strategy to increase efficacy is to make multiple (three or more) applications, particularly if initiated when plants are small (Hoeft et al. 2001; Johnson and Wyse 1991, 1992). Five Pst (10^8 cfu ml^{-1}) applications at weekly intervals, initiated shortly after emergence, killed Canada thistle (Johnson and Wyse 1991). Although multiple applications may achieve better control of Canada thistle shoot growth and may reduce survival, such a strategy is not likely to be widely adopted. An alternative strategy for increasing Pst efficacy on Canada thistle is to increase the amount of tagetitoxin produced in planta by a single application. Disease incidence (apical chlorosis) is caused by the ability of Pst to produce tagetitoxin in planta, which blocks chloroplast biogenesis in developing leaves. It appears that a single Pst application produces a pulse of tagetitoxin which causes apical chlorosis. But after time, presumably because tagetitoxin production is not sustained, the chlorotic leaves re-green, and new growth is not chlorotic. A similar pattern of apical chlorosis followed by re-greening will occur if tagetitoxin is injected into host species (Gronwald and Plaisance, unpublished data; Styer 1982). To improve the efficacy of Pst treatment in reducing Canada thistle growth and survival, tagetitoxin production in planta must be enhanced. One approach would involve collecting Pst strains from various host species and identifying those that exhibit an enhanced capacity to produce tagetitoxin in Canada thistle. An alternative approach would involve enhancing the toxin-producing capacity of a particular Pst strain such as 1-502a used in this study. Toxin production by various plant Pseudomonads is regulated by environmental and nutritional factors (Bender et al. 1999; Li et al. 1998). Little is known about factors regulating tagetitoxin production by Pst. Research conducted with Pst in culture suggests that high nitrogen levels (50 mM nitrate or ammonium) promote tagetitoxin production (Styer 1982). In addition, Durbin (1990) reported that specific amino-containing compounds, which were not identified, triggered tagetitoxin production in culture. Future research needs to identify environmental and nutritional factors that regulate tagetitoxin production by Pst in Canada thistle. Such knowledge may be useful for developing growing conditions in culture that would enhance the toxin-producing capacity of Pst in planta. Alternatively, this knowledge could be used to develop an application formulation that would promote sustained production of tagetitoxin in Canada thistle.

Sources of Materials

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

[1] Seeds, V & J Seed Farms, 14020 Pleasant Valley Road, P.O. Box 82, Woodstock, IL 60098.

[2] Potting medium, SunGro Horticulture, 15831 NE 8th Street, Bellevue, WA 98008.

[3] Organosilicone surfactant, Witco, 777 Old Saw Mill Road, Tarrytown, NY 10591.

[4] Sprayer, Deluxe air spray gun, Northern Tool and Equipment, 1255 Cope Avenue E., Minneapolis, MN 55109.

[5] ALL-SPRAY, 3.8 L, Model 010TV, Vermont American Corp., Chicago, IL 606031.

[6] Two wheel bicycle applicator with a 10-ft boom and five #8004 TeeJet flat fan nozzles (Spraying Systems, Wheaton, IL) spaced at 20-inch intervals. Custom made by Facilities Management, University of Minnesota.

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Literature Cited

Abbas, H. K., D. R. Johnson, B. J. Johnson, and D. L. Wyse. 1995. Effect of *Pseudomonas syringae* pv. *tagetis* on common cocklebur (*Xanthium strumarium* L.). Phytopathology 85:1130.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24:1–15.

Bailey, K. L., S. M. Boyetchko, J. Derby, W. Hall, K. Sawchyn, T. Nelson, and D. R. Johnson. 2000. Evaluation of fungal and bacterial agents for biological control of Canada thistle. Pages 203–208 *in* N. R. Spencer, ed. Proceedings of the 10th International Symposium on Biological Control of Weeds. Bozeman, MT: Montana State University.

Bender, C. L., F. Alarcon-Chaidez, and D. C. Gross. 1999. *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiol. Mol. Biol. Rev. 63: 266–292.

Durbin, R. D. 1990. Biochemistry of non-host-selective phytotoxins. Pages 63–71 *in* R. E. Hoagland, ed. Microbes and Microbial Products as Herbicides. ACS Symposium 439. Washington, DC: American Chemical Society.

Field, R. J. and N. G. Bishop. 1988. Promotion of stomatal infiltration of glyphosate by an organosilicone surfactant reduces the critical rainfall period. Pestic. Sci. 24:55–62.

Gulya, T. J., R. Urs, and E. E. Banttari. 1982. Apical chlorosis of sunflower caused by *Pseudomonas syringae* pv. *tagetis*. Plant Dis. 66:598–600.

Henis, Y., Y. Okon, E. Sharon, and Y. Bashan. 1982. Detection of small numbers of phytopathogenic bacteria using the host as an enrichment medium. Pages 246–247 *in* M. E. Rhodes-Roberts and F. A. Skinner, eds. Bacteria and Plants. New York: Academic Press.

Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. University of California Agricultural Experiment Station Circ. 347. 32 p.

Hoeft, E. V. 1998. Integrated Weed Management for the Control of Canada thistle (*Cirsium arvense*) with *Pseudomonas syringae* pv. *tagetis* and a Weed-competitive Soybean (*Glycine max*) Cultivar. M.S. thesis. University of Minnesota, St. Paul, MN. 60 p.

Hoeft, E. V., N. Jordan, J. Zhang, and D. L. Wyse. 2001. Integrated cultural and biological control of Canada thistle in conservation tillage soybean. Weed Sci. 49:642–646.

Johnson, D. R. and D. L. Wyse. 1991. Use of *Pseudomonas syringae* pv. *tagetis* for control of Canada thistle (*Cirsium arvense*). Proc. N. Cent. Weed Sci. Soc. 46:14.

Johnson, D. R. and D. L. Wyse. 1992. Biological control of weeds with *Pseudomonas syringae* pv. *tagetis*. Proc. N. Cent. Weed Sci. Soc. 47:16.

Johnson, D. R., D. L. Wyse, and K. J. Jones. 1996. Controlling weeds with phytopathogenic bacteria. Weed Technol. 10:621–624.

Kado, C. I. and M. G. Heskett. 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. Phytopathology 60:969–976.

Li, X.-Z., A. N. Starratt, and D. A. Cuppels. 1998. Identification of tomato leaf factors that activate toxin gene expression in *Pseudomonas syringae* pv. *tomato* DC3000. Phytopathology 88:1094–1100.

Lukens, J. H. and R. D. Durbin. 1985. Tagetitoxin affects plastid development in seedling leaves of wheat. Planta 165:311–321.

Mathews, D. E. and R. D. Durbin. 1990. Tagetitoxin inhibits RNA synthesis directed by RNA polymerases from chloroplasts and *Escherichia coli*. J. Biol. Chem. 265:493–498.

Mathews, D. E. and R. D. Durbin. 1994. Mechanistic aspects of tagetitoxin inhibition of RNA polymerase from *Escherichia coli*. Biochemistry 33: 11987–11992.

Neumann, P. M. and R. Prinz. 1974. Evaluation of surfactants for use in the spray treatment of iron chlorosis in citrus trees. J. Sci. Food Agric. 25:221–226.

Rhodehamel, N. H. and R. D. Durbin. 1985. Host range of strains of *Pseudomonas syringae* pv. *tagetis*. Plant Dis. 69:589–591.

Sheikh, T., T. A. Wheeler, P. A. Dotray, and J. C. Zak. 2001. Biological control of woollyleaf bursage (*Ambrosia grayi*) with *Pseudomonas syringae* pv. *tagetis*. Weed Technol. 15:375–381.

Steinberg, T. H., D. E. Mathews, R. D. Durbin, and R. R. Burgess. 1990. Tagetitoxin: a new inhibitor of eukaryotic transcription by RNA polymerase III. J. Biol. Chem. 265:499–505.

Stevens, P.J.G., R. E. Gaskin, S.-O. Hong, and J. A. Zabkiewicz. 1991. Contributions of stomatal infiltration and cuticular penetration to enhancements of foliar uptake by surfactants. Pestic. Sci. 33:371–382.

Styer, D. J. 1982. *Pseudomonas syringae* pv. *tagetis*: Hosts, Symptomatology and Toxin Production. Ph.D. dissertation. University of Wisconsin, Madison, WI. 157 p.

Styer, D. J. and R. D. Durbin. 1982. Common ragweed: a new host of *Pseudomonas syringae* pv. *tagetis*. Plant Dis. 66:71.

Zidack, N. K. and P. A. Backman. 1996. Biological control of kudzu (*Pueraria lobata*) with the plant pathogen *Pseudomonas syringae* pv. *phaseolicola*. Weed Sci. 44:645–649.

Zidack, N. K., P. A. Backman, and J. J. Shaw. 1992. Promotion of bacterial infection of leaves by an organosilicone surfactant: implications for biological weed control. Biol. Control 2:111–117.

Zidack, N. K., B. J. Jacobsen, and P. C. Quimby, Jr. 2000. Biological control of houndstongue (*Cynoglossum officinale*) with the plant pathogen *Pseudomonas syringae* pv. *tagetis* synergized with pelargonic acid. Page 250 *in* N. R. Spencer, ed. Proceedings of the 10th International Symposium on Biological Control of Weeds. Bozeman, MT: Montana State University.

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